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ORIGINAL ARTICLE

Histological, molecular and biochemical detection of renal injury after *Echis pyramidum* snake envenomation in rats

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Abstract Nephrotoxicity is a common sign of snake envenomation. The present work aimed to clarify the effect of intraperitoneal injection of 1/8 LD_{50} and 1/4 LD_{50} doses of *Echis pyramidum* snake venom on the renal tissue of rats after 2, 4 and 6 h from envenomation. Histopathological examination showed intense dose and time dependent abnormalities, including swelling glomerulus and tubular necrosis and damage as well as signs of intertubular medullary hemorrhage at early stages of envenomation. However, at late stages of envenomation by any of the doses under investigation, no intact renal corpuscles were recorded and complete lysis in renal corpuscles with ruptured Bowman's capsules was observed. Immunohistochemistry by immunohistochemical staining was used to test the protein expression of Bax in renal tissue of rats. The result showed that the expression of Bax in renal tissue sections of envenomated rats was increased according to dose and time-dependant manner. The isolation of DNA from the renal cells of envenomed rats pointed out to the occurrence of DNA fragmentation, which is another indicator for renal tissue injury especially after 6 h of 1/4 LD_{50} of *E. pyramidum* envenomation. Oxidative stress biomarkers malondialdehyde and nitrite/nitrate levels, antioxidant parameters; glutathione, total antioxidant capacity and catalase were assayed in renal tissue homogenates. The venom induced significant increase in the levels of malondialdehyde and nitrite/nitrate while the levels of glutathione, total antioxidant capacity and catalase were significantly decreased, especially after 6 h of envenomation. The results revealed that the *E. pyramidum* induced dose and time-dependant significant disturbances in the physiological parameters in the kidney. We conclude that the use of the

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immunohistochemical techniques, the detection of DNA integrity and oxidative stress marker estimations are more specific tools that can clarify cellular injury and could point out to the defense activity of the renal tissue at envenomation.

1. Introduction

Venomous and poisonous snakes are a significant cause of global morbidity and mortality. They are found almost throughout the world, including many oceans and have evolved a variety of highly effective toxins and methods of delivery. Their impact on humans is considerable, most current data suggest that they cause in excess of 3 million bites per year with more than 150,000 deaths, particularly in rural tropical areas (White, 2000).

The pyramid viper (*Echis pyramidum*) is one of the venomous snakes found in Saudi Arabia. It is widely spread in Jazan which according to surveillance, extends from A-Darb city north to Al-Mousam city in the south, near the Saudi-Yemeni borders and from Jazan city west to Fifa and Al-Da'r cities in the east. The pyramid viper has been related with the majority of envenomation cases in the region. The victims of bites who were examined in the local hospitals were suffering from symptoms of nausea and vomiting, swelling at bitten place, low blood pressure, painful bitten site, sweating, bleeding in the area of bite, nose bleeding, fever and unconsciousness (Al-Shammari et al., 2013). Efforts have been made to correlate the toxicity of snake venoms to their enzymatic activities. Owing to the diverse character of the venom and the antagonistic behavior of its different components, it is advantageous to utilize purified venom fractions in place of crude venom for diverse toxicological and pharmacological studies.

Clinical symptoms of *E. pyramidum* envenomation are characterized by highly complex pathophysiological features of local as well as systemic nature. Crude venom of viper *Echis* genus caused renal dysfunction in envenomated Guinea pigs (Warrell, 1993; Salman, 2009). In addition, Al-Asmari et al. (2014) concluded that the acute phase oxidative stress due to *Echis pyramidum* venom (EPV) injection points toward the importance of an early antioxidant therapy for the management of snake bites. From this point, the aim of our study was to investigate the effect of different doses of EPV at different times on the renal tissue of rats.

2. Materials and methods

2.1. Echis pyramidum venom (EPV)

Specimens of EPV were captured in the Saudi Arabian desert and maintained at 28 ± 2 °C on a diurnal cycle of 7 h light/17 h dark. Each snake was fed one 20–30 g mouse every two weeks with water ad libitum. Venom was milked from the snakes as described by Al-Saleh et al. (1994), lyophilized and stored at −20 °C until used then reconstituted in 1× phosphate-buffered saline (PBS) prior to use. The approximate median lethal dose (LD_{50}) of the crude venom was found to be 5.06 mg/kg rat (Al-Shammari et al., 2013).

2.2. Experimental design

The study was performed using 42 healthy male Albino Wistar rats, weighting 120 ± 10 g obtained from the breeding unit of Holding Company for Biological Products and Vaccines, "VACSERA", Cairo-Egypt. They were kept under standard laboratory conditions and fed on normal basic diet. They were acclimatized to the lab conditions for a week prior to the experiment. Rats were divided into 3 groups:

Control group (n = 6): Rats were intraperitoneally (i.p.) injected with 0.1 ml PBS solution, and sacrificed after 6 h from the injection.

1/8LD50-Envenomed group (n = 18): Rats were i.p. injected with 0.1 ml saline solution containing 0.6325 mg venom /kg body weight of the rat. The rats were subdivided into three subgroups (six rats each) sacrificed after 2 h, 4 h and 6 h from envenomation respectively.

1/4LD50-Envenomed group (n = 18): Rats were i.p. injected with 0.1 ml saline solution containing 1.265 mg venom /kg body weight of the rat. This group was subdivided into three subgroups (six rats each) sacrificed after 2 h, 4 h and 6 h from envenomation respectively.

2.3. Histological preparations

After abdominal dissection samples from the kidney were removed from each rat and washed in saline solution then fixed in 10% neutral buffered formalin for 24 h. Following the routine procedure of the paraffin method, kidney samples were processed up to paraffin blocks. Paraffin sections (5 μ thick) were stained with hematoxylin and eosin for regular histological investigation. The preparations obtained were visualized using a light microscopy at a magnification of 400×.

2.4. Immunohistochemical studies

Bax protein products were detected by specific monoclonal antibodies. From each kidney block, 4-pm-thick sections were cut on Neoprene-coated slides. The immunostaining was performed using the avidin–biotin complex (ABC) method and an automatic autostainer (CODE-ON Immuno/DNA slide stainer: Biotek solution, Santa Barbara, CA). Slides were deparaffinized and blocked for endogenous peroxidase with 1.75% hydrogen peroxide in methanol for 20 mm, antigen retrieval for 15 mm using Biogenex antigen retrieval citra solution in 90 °C water bath for 30 mm. The slides were allowed to cool for 20 min before continuing. Slides were then blocked by normal horse serum for 5 mm at 37 °C. The monoclonal antibody was applied overnight in humid medium at room temperature followed by the Biotinylated secondary antibody for 15 min at 37 °C and the ABC complex for 15 min at

37 °C (Vectastain Elite ABC Kit; Vector Laboratories, Burlingame, CA). Diaminobenzidine (DAB) was applied for 20 min at room temperature as chromogen, slides were counterstained with hematoxylin, dehydrated, and covered by coverslips. In negative control slides, the same system was applied with replacement of the monoclonal antibody by diluted normal bovine serum. Bax immunostaining was performed using polyclonal rabbit-anti-human (A3533 Ig fraction; Dako, Glostrup, Denmark) at a dilution of 1:50 (El Nahas, 1992).

2.5. DNA fragmentation assay using agarose gel electrophoresis

As a measure of apoptotic DNA fragmentation, the presence of DNA ladder was determined according to Wlodek et al. (1991). Extraction of DNA was done according to the method of Aljanabi and Martines (1997). 20 mg of renal tissue in eppendorf tubes was lysed with 600 µl buffer (50 mM NaCl, 1 mM Na2EDTA, 0.5% SDS, PH 8.3) and gently shaken. The mixture was incubated overnight at 37 °C then, 20 µl of saturated NaCl was added to the sample, shaken and centrifuged at 12,000 rpm for 10 min. The supernatant was transferred to new eppendorf tubes and then DNA precipitated by 600 µl cold isopropanol. The mix was inverted several times till fine fibers appeared, and then centrifuged for 5 min. at 12,000 rpm. The supernatant is removed and the pellets were washed with 500 µl 70% ethyl alcohol then centrifuged at 12,000 rpm for 5 min. After centrifugation the alcohol was decanted or tipped out and the tubes were plotted on Whatman filter paper to dry. The pellets were resuspended in 50 µl or appropriate volume of TE buffer (10 mM Tris, 1 mM EDTA, PH 8). The resuspended DNA was incubated for 30–60 min with loading mix (RNase + loading buffer) and then loaded into the gel wells.

A gel was prepared with 2% agarose containing 0.1% ethidium bromide (200 µg/ml). The DNA samples were mixed with loading buffer (0.25% bromophenol blue, 0.25% xylene cyanole FF and 30% glycerol) and loaded into the wells (20 µl of DNA/lane) with a standard molecular-sized ladder marker (Pharmacia Biotech., USA). The gel was electrophoresed at a current of 50 mA for 1.5 h using the submarine gel electrophoresis machine. The DNA was visualized and photographed with illumination under UV light.

2.6. Biochemical studies

Pieces of kidneys were homogenized immediately to give 50% (w/v) homogenate in ice–cold medium containing 50 mM Tris–HCl, pH 7.4. The homogenates were cold centrifuged at 500×*g* for 10 min. The supernatant (10%) was used for the various biochemical determinations.

2.6.1. Kidney function tests

The urea was estimated in the renal homogenate by Wybenga et al. (1971) using the commercially available kit. Briefly urea was condensed with diacetyl monoxime in an acidic medium to form a red colored complex. In addition, the creatinine was estimated by modified Jaffe's method (Chromy et al., 2008) although uric acid was measured in the renal homogenate according to the method of Fossati et al. (1980).

2.6.2. Oxidative stress and enzymatic antioxidant markers

Lipid peroxidation was assayed colorimetrically in the renal homogenates according to the method described by Ohkawa et al. (1979). Lipid peroxidation determined by using 1 ml of trichloroacetic acid 10% and 1 ml of thiobarbituric acid 0.67% and were then heated in a boiling water bath for 30 min. Thiobarbituric acid reactive substances were determined by the absorbance at 535 nm and expressed as malondialdehyde (MDA) formed.

The assay of nitrite/nitrate, as an indirect measure of nitric oxide production, content in renal homogenates was done according to the method described by Green et al. (1982). In an acid medium and in the presence of nitrite the formed nitrous acid diazotized sulfanilamide was coupled with N-(1-naphthyl) ethylenediamine. The resulting azo-dye had a bright reddish–purple color which could be measured spectrophotometrically at 540 nm.

The renal glutathione (GSH) level was determined according to Ellman (1959). The method was based on the reduction of Elman's reagent (5, 5′ dithiobis (2-nitrobenzoic acid) "DTNB") with GSH to produce a yellow compound. The reduced chromogen was directly proportional to GSH concentration and its absorbance was measured at 405 nm.

Catalase (CAT) activity was estimated according to the method of Aebi (1984), CAT reacts with a known quantity of H_2O_2. The reaction is stopped after exactly 1 min with CAT inhibitor. In the presence of peroxidase, the remaining H_2O_2 reacts with 3,5-dichloro-2-hydroxybenzene sulfonic acid and 4-aminophenazone to form a chromophore with color intensity inversely proportional to the activity of catalase in the original sample.

Total antioxidant capacity (TAC) in different areas of the brain was assayed by the colorimetric technique using kit of Biodiagnostic (Egypt) according to Koracevic et al. (2001) method.

The total protein content of the homogenized kidney was determined by the method of Lowry et al. (1951) using bovine serum albumin as a standard. Lactate dehydrogenase (LDH) was measured by using *Biosystems* Kit, *Barcelona*, *Spain*. LDH catalyzes the reduction of pyruvate by NADH reagent, to form lactate and NAD^+. The catalytic concentration is determined from the rate of decrease of NADH, measured at 340 nm.

2.7. Statistical analysis

Data are presented as means ± standard error of the mean (SEM) and statistically analyzed using ANOVA test (version 17.00). Significance was set at the level of $P < 0.05$ versus control.

3. Results

3.1. Histological observations

Light microscopy showed that control kidney section had a normal morphology and no changes in the renal parenchyma (Fig. 1A). A venom dose at 1/8 LD50 after 2 h injection induced tubular cell acidophilia, indicating cell damage (Fig. 1B); in addition, peritubular capillary congestion after

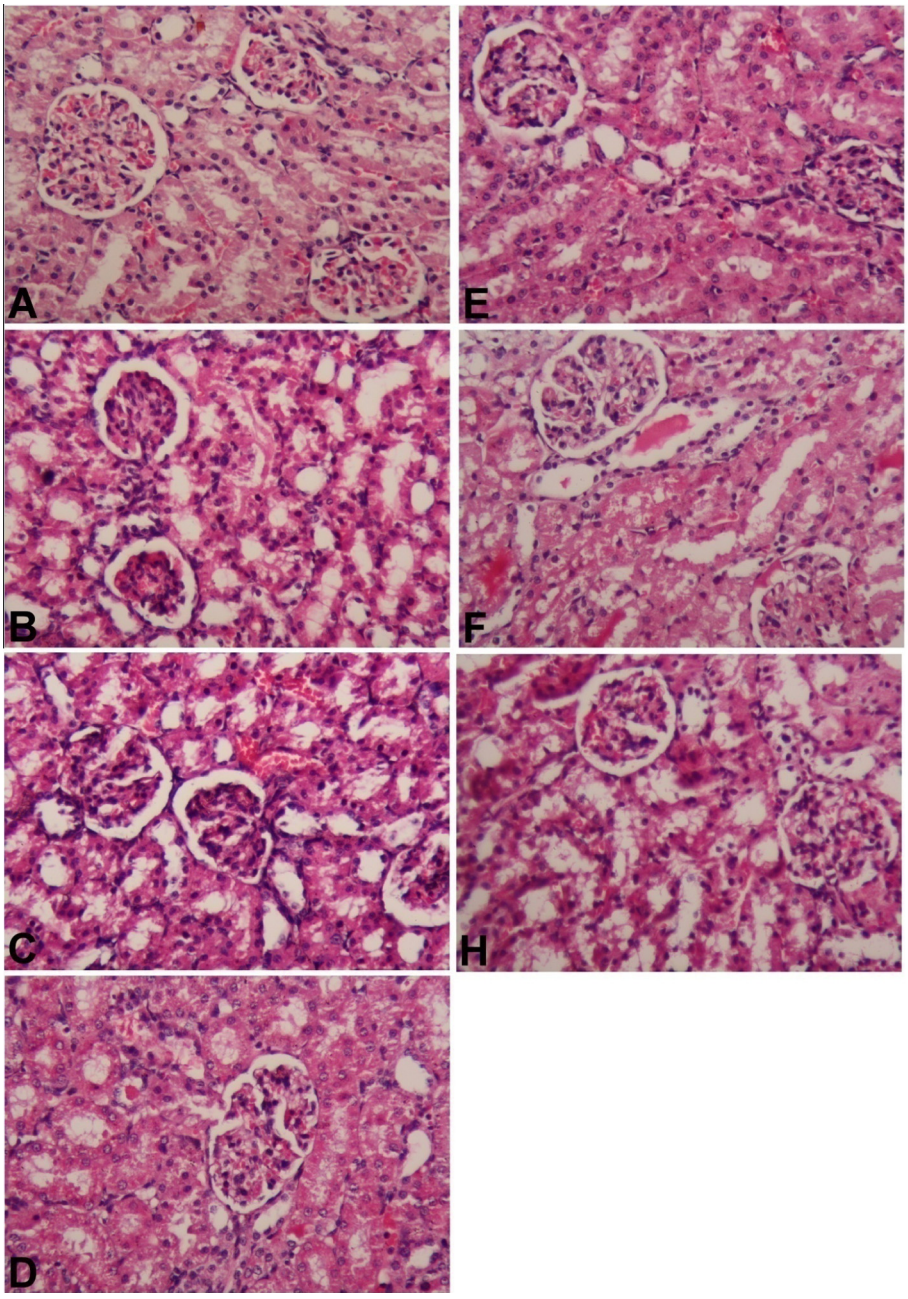

Figure 1 (A) Kidney of rat in the control group, showing intact architecture of the glomerulus and renal tubules (B, C and D) kidney of rat 2 h, 4 h and 6 h post 1/8 LD_{50} EPV, respectively and (E, F and H) kidney of rat 2 h, 4 h and 6 h post 1/4 LD_{50} EPV, respectively, showing swelling glomerulus and degenerative renal tubules. Sections were stained with HE, 400×.

the 4th and 6th h of the venom injection was found (Fig. 1C & D) respectively. Moreover, significant degenerative changes were seen in the proximal tubules; in the form of cytoplasmic vacuolations (Fig. 1B & C), and hydropic degeneration with enlarged nuclei in some cells (Fig. 1D).

However, the glomeruli showed significant and marked changes as a result of the injection of 1/8 LD50 and 1/4 LD50 doses at all the experimental time intervals where partially destroyed glomerular capillaries with dilated Bowman's space were observed (Fig. 1A: H); besides, the glomerular tuft was noticed after the 4 h of the envenomation (1/8LD50).

Likewise, the renal tissue after 1/4 LD50 EPV injection on the 2nd, 4th and 6th h (Fig. 1E, F and H respectively) showed severe degenerative changes in the form of hydropic degeneration with enlarged nuclei in some cells of the proximal tubules. These changes consisted of a loss of proximal brush border, cytoplasmic vacuolation. In some areas, there was disruption of both the peritubular capillaries and tubular walls, indicating

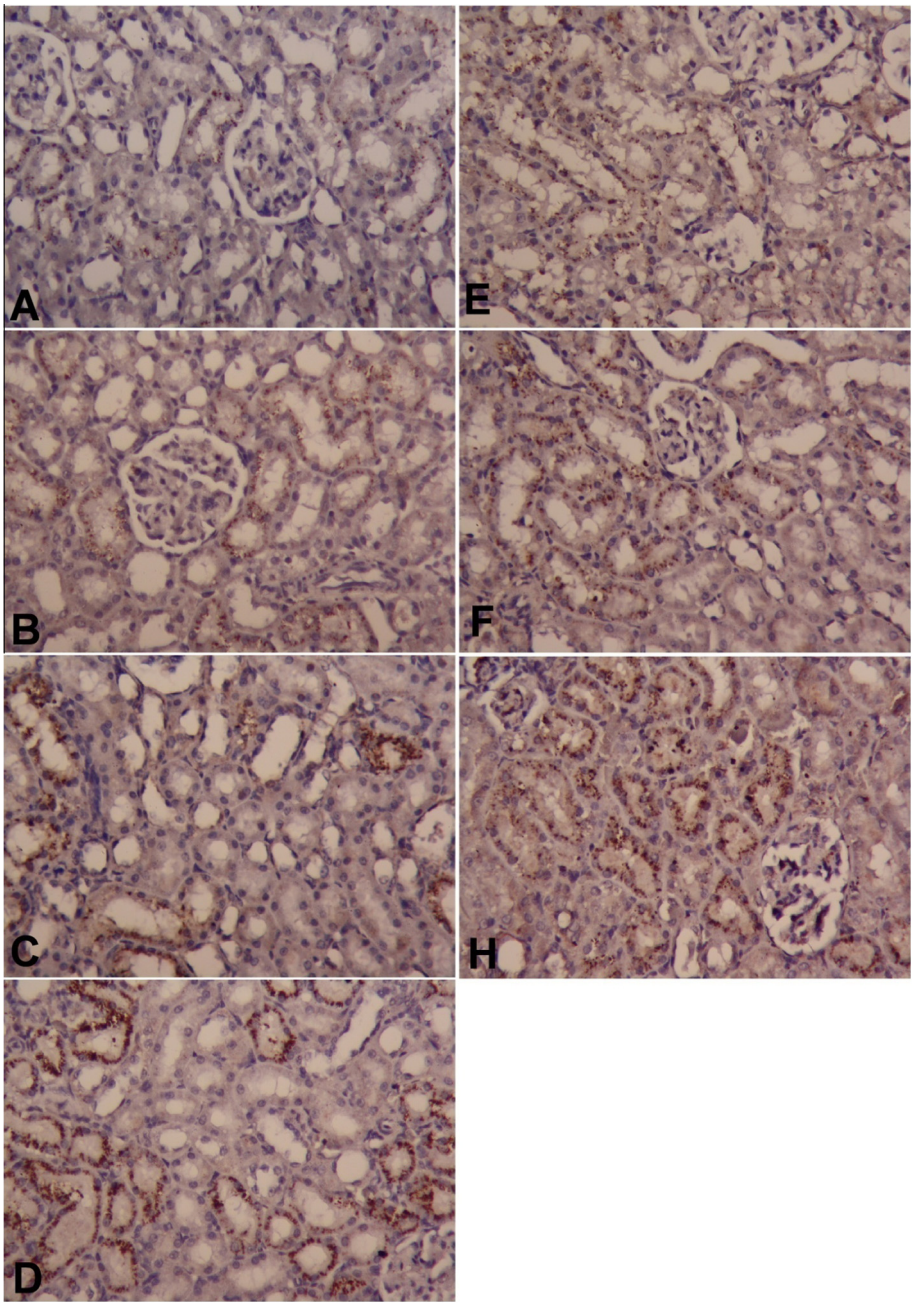

Figure 2 The expression of Bax in renal tissue of rats in normal and treated groups. (A) Section of kidney of normal rat shows negative reaction, (B–D) sections of kidney of rat 2 h, 4 h and 6 h post 1/8 LD_{50} EPV, respectively and (E–H) sections of kidney of rat 2 h, 4 h and 6 h post 1/4LD_{50} EPV, respectively showing (immunohistochemistry staining, 400×).

hemorrhage. Also, sloughing of lining epithelium of tubules and tubular necrosis were observed.

3.2. Immunohistochemical observations

The renal control tissue showed Bax negative reaction (Fig. 2A). On the contrary, some cells of tubules stained positively for Bax and the staining was visibly increased in damaged tubules (Fig. 2B–D) due to the influence of 1/8 LD50 EPV injection on the 2nd, 4th and 6th h respectively.

The Bax positive reaction in the cytoplasm of tubular epithelial cells of the 1/4 LD50 envenomated rats was more intensified (Fig. 2E, F and H) at all selected time intervals. In addition, the positive reaction of Bax was clear in the glomeruli (Fig. 2F and H) after injection of the EPV on the 4th and 6th h.

3.3. DNA fragmentation assay results

The EPV-induced apoptotic DNA fragmentation in kidneys of envenomated rats was clearly indicated on the agarose gel as detected by ethidium bromide fluorescence (Fig. 3). In the DNA of normal kidney tissue no ladder was observed (Fig. 3, lane 1), and a genomic DNA ladder formation was observed when rats were injected with crude EPV at 1/8 and

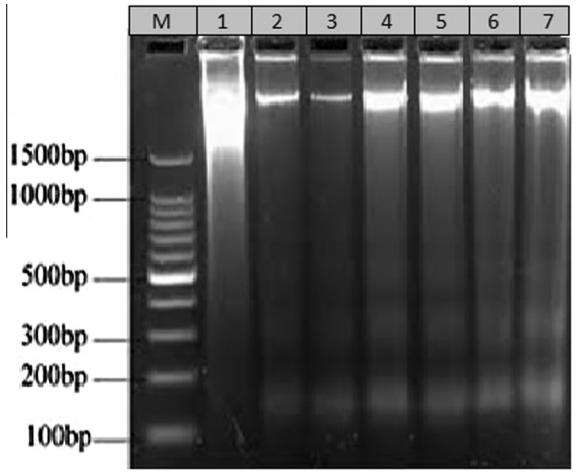

Figure 3 Agarose gel electrophoresis photograph of DNA extracted from kidney tissue of normal and EPV injected rats. (Lane 1, DNA profile of normal rat kidney), (lane 2–4, DNA profile of 2 h, 4 h and 6 h post 1/8LD_{50} EPV injection), (lane 5–7, DNA profile of 2 h,4 h and 6 h post 1/4LD_{50} EPV injection) and (lane 8, DNA ladder).

1/4 LD_{50} (Fig. 3, lane 2–7). EPV indicated dose and time-dependent, typical apoptotic fragmentation of the DNA. The degradation of DNA into oligonucleotide fragments was maximal after 6 h post injection with 1/8 LD_{50} and after 2, 4 and 6 h post 1/4 LD_{50} injection of EPV, confirming the induction of apoptosis by EPV injection (Fig. 3, lanes 4, 5, 6 and 7, respectively).

3.4. Biochemical results

The represented data in Fig. 4 show the levels of urea, uric acid and creatinine in the renal homogenates of the control normal and EPV injected rats. Envenomation by different doses of EPV (1/8LD_{50} and 1/4LD_{50}) resulted in a significant elevation in the urea level at all studied time intervals as compared to its control group.

Moreover, 1/8LD_{50} dose of EPV injection caused a significant increase in the uric acid level on the 2nd h, while, a significant reduction was recorded in the uric acid level on the 4th h and 6th h ($P < 0.05$) versus its control normal group. On the other hand, 1/4LD_{50} dose of the venom induced a significant decrease in the level of uric acid. Otherwise, the injection induced a significant increment on the 4th h and 6th h as compared to control value.

Creatinine level showed a non-significant change on the 2nd and 6th h and a significant increase ($P < 0.05$) on the 4th h as a result of 1/8 LD_{50} dose envenomation as compared to control value. Meanwhile, the envenomation of 1/4 LD_{50} dose to rats induced a significant increment in creatinine level all over the experimental time intervals as shown in Fig. 4.

In the present work, rats injected with crude EPV at 1/8 and 1/4 LD_{50} doses for the 2nd h, 4th h and 6th h caused different changes of the selected biochemical parameters. A non-significant change was recorded in the MDA level on the 2nd h due to the venom injection at a dose level 1/8 LD_{50}. A significant increase was noticed on the 4th h and 6th h. The injection of 1/4 LD_{50} dose increased the MDA level significantly ($P < 0.05$) all over the experimental time intervals (Fig. 5). Similarly, the nitrite/nitrate level showed a significant elevation resulting from the envenomation at all the investigated doses and time intervals in the renal homogenate of rats. On the contrary, the i.p. injection of EPV (1/8LD_{50} and 1/4LD_{50} doses) to rats indicated a significant reduction in GSH level of the renal homogenate on the 2nd h, 4th h and 6th h.

Fig. 6 shows a highly significant decrease at $P < 0.05$ in the CAT activity and TAC of the renal tissue homogenate at all time intervals and at the doses under investigations as compared to its corresponding control.

With respect to the values of the total protein content recoded on the 2nd h, 4th h and 6th h; there was an increment in the content of total protein on the 2nd h as a result of the i.p. injection of the crud EPV (1/8 LD_{50}); meanwhile a non-significant change was observed on the 4th h and 6th h. Moreover, the effect of 1/4 LD_{50} of the EPV induced a significant rise in the total protein content on the 2nd h and 4th h (Table 1).

Table 2 represents the effect of the crude venom (1/8LD50 and 1/4LD50) on the LDH activity in the renal homogenate; there were significant elevations starting from the 4th h of the injection till 6th h as compared to its control value.

4. Discussion

Relatively little work has been done on the toxicological effects of crude EPV. Okuda et al. (2001) purified novel disintegrins,

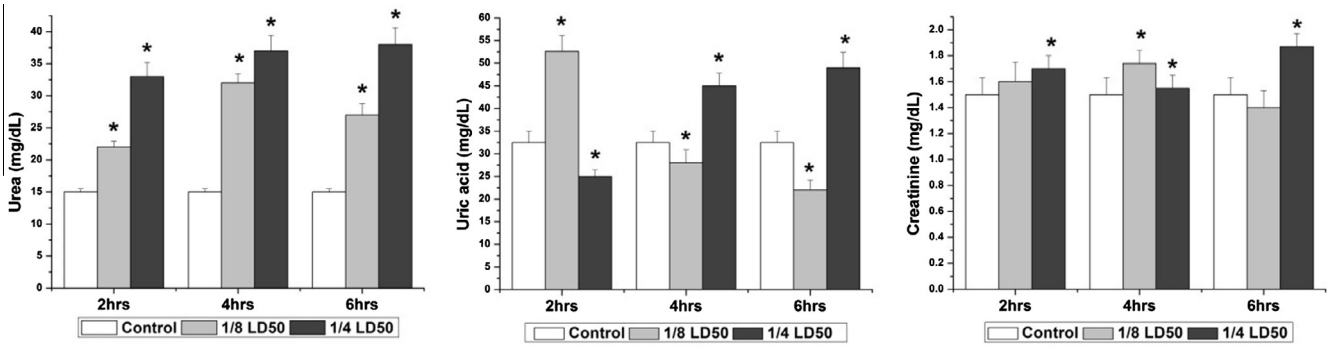

Figure 4 The changes of urea, uric acid and creatinine levels (mg/dl) in renal homogenate as a result of 1/8 and 1/4 LD_{50} of EPV i.p. injection at the 2nd h, 4th h and 6th h in adult male albino rat, *: Significant against vehicle control group at $P \leqslant 0.05$.

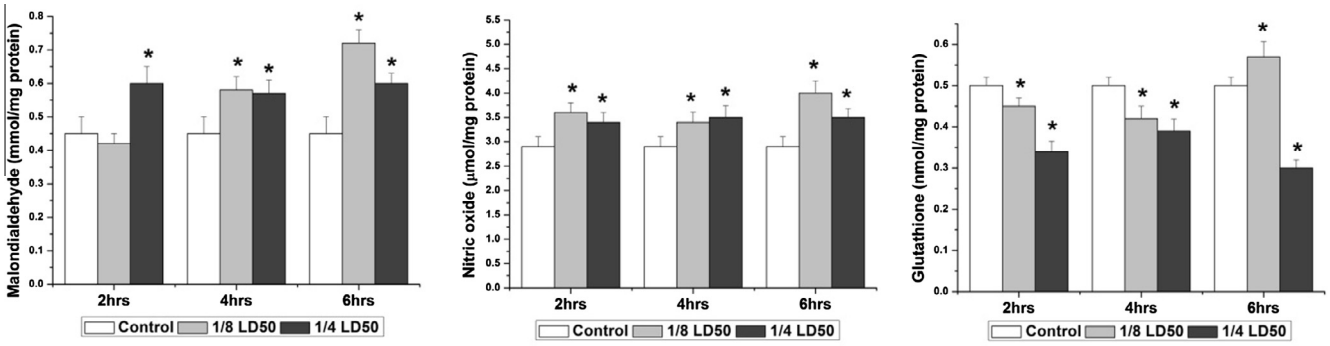

Figure 5 Malondialdhyde (mmol/mg protein), nitrite/nitrate (μmol/mg protein) and GSH (nmol/mg protein) levels in renal homogenate after i.p. injection (1/8 and 1/4 LD_{50}) of EPV on the 2nd h, 4th h and 6th h in adult male albino rat, *: Significant against vehicle control group at $P \leqslant 0.05$.

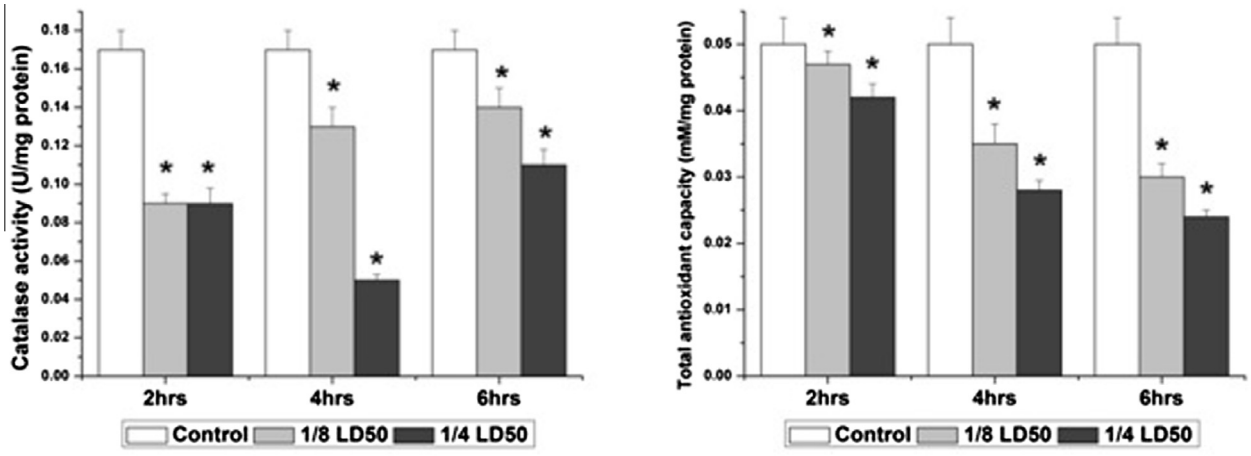

Figure 6 The intraperitoneal injection of EPV at a dose level of 1/8 and 1/4 LD_{50} induced alterations in CAT (U/mg protein) activity and TAC (mM/mg protein) level in renal homogenate of adult male albino on the 2nd h, 4th h and 6th h, *: Significant against vehicle control group at $P \leqslant 0.05$.

Table 1 The effect of 1/8 and 1/4 LD_{50} of crude EPV i.p. injection on the 2nd h, 4th h and 6th h on total protein level (mg/g tissue) in renal homogenate of rats.

Parameter	Total protein (mg/g tissue)		
Groups	2 h	4 h	6 h
Control	6.72 ± 0.32	6.72 ± 0.32	6.72 ± 0.32
1/8 LD50	9.73 ± 0.43*	7.15 ± 0.28	6.53 ± 0.41
1/4 LD50	7.80 ± 0.57*	8.66 ± 0.42*	6.60 ± 0.24

Values are means ± SEM.
* Significant against vehicle control group at $P \leqslant 0.05$, $n = 6$.

Table 2 The lactate dehydrogenase activity (U/g tissue) showed alterations in the rat's renal homogenate envenomated with crude EPV (1/8 and 1/4 LD_{50}) at different time intervals.

Parameter	LDH (U/g tissue)		
Groups	2 h	4 h	6 h
Control	199.1 ± 10.4	199.1 ± 10.4	199.1 ± 10.4
1/8 LD50	188.8 ± 15.7	229.9 ± 19.3*	311.8 ± 11.4*
1/4 LD50	203.3 ± 13.3	250.3 ± 15.8*	351.4 ± 19.7*

Values are means ± SEM.
* Significant against vehicle control group at $P \leqslant 0.05$, $n = 6$.

the platelet aggregation inhibitors pyramidin A and B from the venom of *E. pyramidum*. Al-Asmari et al. (2006) studied the effect of crude venom on time-course of lipid peroxidation in different organs of mice. El-Missiry et al. (2010) investigated the effect of crude venom on the activities of certain serum enzyme of mice. Wahby et al. (2012) isolated purified hemorrhagic metalloproteinase enzyme from EPV and studied its hemorrhagic activities in the skin of rabbit. Most recently, Conlon et al. (2013) isolated [Ser49] phospholipase A_2 from the venom of the saw-scaled vipers *Echis ocellatus*, *Echis pyramidum leakeyi*, *Echis carinatus sochureki*, and *Echis coloratus* and observed their cytotoxic activity against human non-small cell lung adenocarcinoma A549 cells. So, this work was undertaken to study the effect of EPV on histological, molecular changes and on oxidative stress status in renal homogenates.

Snake venom components, especially those of viper venoms, activate, inhibit or liberate enzymes by destroying cellular organelles (Abdel-Nabi et al., 1997; Marsh et al., 1997).

The different toxic effects of viper venoms are due to their proteolytic and lipolytic enzymes (Tan and Ponnudurai, 1990). Common initial signs of envenomation are hypoglycemia (Abu-Sinna et al., 1993), general metabolic disturbance (Mahmoud, 1983), muscular dystrophy (Mohamed and Khaled, 1966), nephrotoxicity (Ickowicz et al., 1966) and cytotoxicity (Bertke and Atkins, 1961).

The local effects of the snake bite include, edema, hemorrhage, dermonecrosis and myonecrosis (Chippaux et al., 1991; Tu, 1996; Shashidhara-Murthy et al., 2002; Cher et al., 2005) and in agreement with these results, the current study has shown marked histological changes in the renal tissue in the form of swelling glomerulus, tubular necrosis and damage as well as signs of intertubular medullary hemorrhage at early stages of envenomation. However, at late stages of envenomation by any of the doses under investigation, no intact renal corpuscles were recorded; they were completely lysed with ruptured Bowman's capsules after 6 h 1/8 and 1/4 LD_{50} crude venom injection. Renal failure can be expected after envenomation by vipers with a serious course of intoxication. The underlying mechanisms are, besides glomerular hypofiltration by bleeding, decrease of intravascular volume by extravasation, microthrombi formation within consumption coagulopathy and vasoconstriction and direct nephrotoxicity of venom constituents: enzymatic destruction of renal marrow and renal tubules (Warrell, 1995).

Apoptosis is a basic biological phenomenon that could occur in all cells including the histiocytes. It is a kind of special suicide which occurs in response to different stimulation or diseases. Through this, the body maintains physiological balance by eliminating injured, aged and mutational cells (Hu and Gao, 2005). Bax ''proapoptotic antigen'' is recognized as a modulator of the apoptotic program event; its relative level determines the fate of cells (Yang et al., 2001). Moreover, Pierce et al. (2011) deduced that *E. species* venom promotes apoptosis as a part of their pathological mechanisms. These findings are in agreement with our results. Thomas et al. (1998) indicated that apoptosis of renal cells might contribute to the progression of tubular atrophy.

Our findings confirm that EPV envenomation to rats promoted the DNA fragmentation; these results are in agreement with Chethankumar and Srinivas (2014); who indicated that the *Naja haja* induced the DNA fragmentation. DNA damage leads to the accumulation of p-53 protein in the cell and inhibition of the cell cycle. P53 stimulates the production of BAX protein, which is capable of opening the mitochondrial channels from which cytochrome C is released (Pedrycz et al., 2014).

The i.p. injection of EPV (1/8LD50 & 1/4 LD50) to rats resulted in significant disturbances in the renal functions (urea, uric acid and creatinine) in the present work. These results go hand in hand with those of Abdel-Nabi (1993); Al-Jammaz (2001); Salman (2009); El-Missiry et al. (2010); Al-Shammari et al. (2013).

El-Missiry et al. (2010) reported that the effect of the LD50 of native EPV on the kidney and renal functions induced a highly significant increase in urea and creatinine levels compared to the normal control. In addition, Al-Jammaz (2001) and Salman (2009) found that the envenomation of experimental animals with viper snake venom increased the levels of serum urea and creatinine while the level of serum uric acid decreased. The precise mechanisms whereby the venoms cause reduction of serum uric acid level are not fully known.

However, Abdel-Nabi (1993) attributed the significant elevation in serum urea to an increase of nitrogen retention and/or due to corrupted renal function this was concomitant to a significant increase in serum creatinine levels as well. The increase in these values is used as an indicator of renal failure. Injection of sublethal dose of *E. carinatus* venom caused significant disturbances in the renal functions through severe necrosis of kidney tubules and nephrotoxic action (Abdel-Nabi and Rahmy, 1992).

Abdel-Aal (1998) reported that viper venom of *Cerastes cerastes cerastes* increased serum creatinine and urea significantly, 30 min following injection, and that this effect persisted for up to 7 days, indicating renal failure. Also, Tu (1991) and Merchant et al. (1989) reported that renal diseases caused by various snake venoms were characterized by raised urea, creatinine and potassium in oliguric patients. The disturbance in these values is used as an indicator of renal failure and impairment of the excretory function of the kidney which was ascribed to the nephrotoxic effect of venom.

In this study, the envenomated rats with 1/8 and 1/4 LD_{50} of EPV after 2nd h, 4th h and 6th h showed a highly significant increase in lipid peroxidation and nitrite/nitrate levels as compared with the control group. However, EPV injection induced a highly significant reduction in the GSH level, CAT activity and the TAC level after the three selected time intervals with the 2 tested doses. Oxidative stress may be a result of excessive reactive oxygen species generation or failure of the cellular antioxidant system. High dose of EPV induced an elevation of oxidative stress indicators as nitric oxide and lipid peroxidation in renal tissue after 6 h post dosing (Al-Asmari et al., 2006; 2014). Glutathione is widely distributed tripeptide and found mainly in the cell cytosol (Mitchell and Jollow, 1975). Glutathione is the cell's natural antioxidant, which destroys free radicals formed in cells. This plays a crucial role in the detoxification process. Our results were supported by the previous interpretation of the consequences of the GSH deficiency and decreased CAT activity which causes oxidant damage and greater lipid peroxidation which in turn lead to cell damage (Wang et al., 2000; Scholz et al., 1997; Bouchard et al., 2000; Al-Asmari et al. (2014)).

The total protein content in the renal tissue in our investigations showed a significant increase at the beginning of EPV injection followed by a non-significant decrease on the 6th h of the two selected doses of envenomation. Al-Shammari et al. (2013) indicated that the injection of EPV to male mice exhibited potent disturbances in the total plasma protein. It may be attributed to the presence of proteolytic enzymes (proteases) in viper venoms. Al-Saleh (1997) reported that the crude *Bitis arietans* and three of its purified protein fractions showed caseinolytic activity. Wahby et al. (2012) isolated and purified hemorrhagic metalloproteinase from three viper venom including *E. pyramidum* showing strong proteolytic activity.

Moreover, the reduced levels of total protein could be due to disturbances in renal functions as well as hemorrhages in some internal organs. In addition, increasing in vascular permeability and hemorrhages in vital organs due to the toxic action of various snake venoms (Meier and Stocker, 1991; Marsh et al., 1997). In addition, Meier and Stocker (1991)

suggested that viper bites lead to acute nephropathy, where, Ismail et al. (1996) speculated that the tissue distribution of the venom showed the highest uptake in the kidney. Such increased vascular permeability, together with, renal damage would further aggravate the accompanying hypoproteinemia and hypoalbuminaemia (Salman, 2009). However, Tilbury et al. (1987) reported acute renal failure characterized by vascular lesions and tubular necrosis in the renal cortex following various snake bites.

With respect to LDH activity in the renal tissue of the envenomated rats; a significant elevation was recorded all over experimental time intervals of the two selected doses under investigations. Our result was in agreement with Fernando et al., 1989; Aguiyi et al., 2001; Al-Sadoon et al., 2013.

Aguiyi et al. (2001) reported an elevation in the LDH activity following administration of *E. carinatus* venom. Also, Fernando et al. (1989) concluded that *Bothrops asper* venom increased LDH significantly; the highest peak being observed at 6 h. Al-Sadoon et al. (2013) deduced that the injection of *Cerastes cerastes gasperetti* crude venom (LD50) resulted in a highly significant elevation in the LDH activity of renal tissue.

Shaban and Hafez (2003) reported that the LDH is one of the enzyme markers of such injured tissues by a snake envenomation also, the authors concluded that the i.p. injected snake venom caused a marked decrease of serum LDH activity, attributed the reduction of LDH activity to renal damage as well as to the inhibition of their activities caused by the venom as had been suggested by Mohamed et al. (1981).

5. Conclusion

It is clear from this study that, EPV has drastic toxic effects on rat renal tissue as represented by the observed histopathological, molecular and biochemical changes. So our future goal is to characterize the relative role of some medicinal plants and natural products on neutralizing or modulating these toxic effects of the crude EPV.

Conflict of interest

The authors declare that there are no conflicts of interest.

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